Our Ref.: 427.098

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: :
POITOUT et al : Examiner:
Serial No.: 10/550,122 :
Filed: July 7, 2000 : Group:
For: IMIDAZOPYRIDINE ... AGONISTS :

## DECLARATION

Sir:

Christophe Thurieau hereby deposes and says as follows:

That he has been employed by Ipsen Group since October 1996 and is the Vice President of Research of the said company.

That the following tests were conducted under his supervision and he has reviewed the following data which are deemed to accurately reflect the activity of 160 different imidazopyridine derivatives on the melanocortin receptor sub-type 4.

## TEST DATA

The affinity on the melanocortin receptor sub-type 4 of imidazopyridine derivatives of the formula according to the present invention was measured according to procedures analogous to those described hereafter for the MC4 receptors.

The affinity is determined by measuring the inhibition of the binding of [$^{125}$I]-[Nle$^4$, D-Phe$^7$]-α-MSH to membrane preparations of transfected CHO-K1 cells.

CHO-K1 cells expressing rat MC4 receptors in a stable fashion are cultured in an RPMI 1640 medium containing 10% of fœtal calf

serum, 2 mM of glutamine, 100 U/ml of penicillin, 0.1 mg/ml of streptomycin and 0.5 mg/ml of G418. The cells are collected with 0.5 mM of EDTA and centrifuged at 500 g for 5 minutes at 4°C. The pellet is re-suspended in a phosphate buffered saline (PBS) medium and centrifuged at 500 g for 5 min at 4°C. The pellet is re-suspended in a Tris 50 mM buffer medium at pH 7.4 and centrifuged at 500 g for 5 minutes at 4°C. The cells are lysed by sonication and centrifuged at 39,000 g for 10 minutes at 4°C. The pellet is re-suspended in Tris 50 mM buffer medium at pH 7.4 and centrifuged at 50,000 g for 10 minutes at 4°C. The membranes obtained in this last pellet are stored at -80°C.

Measurement of the competitive inhibition of the binding of [$^{125}$I]-[Nle$^4$, D-Phe$^7$]-α-MSH to the MC4 receptors is carried out in duplicate using polypropylene 96-well plates. The cell membranes (20 µg of proteins/well) are incubated with [$^{125}$I]-[Nle$^4$, D-Phe$^7$]-α-MSH (0.15 nM) for 90 minutes at 37°C in a 50 mM Tris-HCl buffer medium, pH 7.4, comprising 0.2% of bovine serum albumin (BSA), 5 mM of $MgCl_2$, and 0.1 mg/ml of bacitracin.

The bonded [$^{125}$I]-[Nle$^4$, D-Phe$^7$]-α-MSH is separated from the free [$^{125}$I]-[Nle$^4$, D-Phe$^7$]-α-MSH by filtration through GF/C glass fibre filter plates (Unifilter, Packard) pre-impregnated with 0.1% of polyethylenimine (P.E.I.), using a Filtermate 196 (Packard). The filters are washed with 50 mM Tris-HCl buffer, pH 7.4 at 0-4°C and the radioactivity present is determined using a counter (Packard Top Count).

The data are analyzed by computer-assisted non-linear regression (MDL) and the values of the inhibition constants (Ki) are determined.

## CONCLUSION

The results as set forth in the tables attached hereto and incorporated herein by reference indicate that the 160 tested compounds exhibit a measurable activity on the melanocortin receptor sub-type 4, below 3.5 µM for all compounds, below 1 µM and even below 250 nM for many of them.

The effects of the melanocortins are mediated by a family of membrane receptors specific to seven transmembrane domains and coupled to the G proteins. If the specific functional roles of each of the five sub-types of receptors are not totally explained, the treatment of pathological disorders or diseases can be associated with an affinity for certain sub-types of receptors.

Thus, the treatment of nutritional disorders has been associated with MC3 and MC4 receptors as discussed by LinksFan et al., Huszar et al. and Dhillo et al. (LinksFan W, Boston BA, Kesterson RA, Hruby VJ, Cone RD, Role of melanocortinergic neurons in feeding and the agouti obesity syndrome, Nature, 1997 Jan 9, 385(6612):165-8 ; Huszar D, Lynch CA, Fairchild-Huntress V, Dunmore JH, Fang Q, Berkemeier LR, Gu W, Kesterson RA, Boston BA, Cone RD, Smith FJ, Campfield LA, Burn P, Lee F, Targeted disruption of the melanocortin-4 receptor results in obesity in mice, Cell, 1997 Jan 10, 88(1):131-41 ; Dhillo WS, Bloom SR, Hypothalamic peptides as drug targets for obesity, Curr Opin Pharmacol., 2001 Dec;1(6):651-5.).

Other indications associated with the activation of MC3 and MC4

receptors are:

- sexual activity disorders as discussed by Wessells et al. and Andersson et al.(Wessels H, Levine N, Hadley ME, Dorr R, Hruby V, Melanocortin receptor agonists, penile erection, and sexual motivation: human studies with Melanotan II, Int J Impot Res. 2000 Oct 12, Suppl 4:S74-9; Andersson KE, Hedlund P, New directions for erectile dysfunction therapies, Int J Impot Res., 2002 Feb 14, Suppl 1:S82-92);

- neuropathic pain as discussed by Vrinten et al. (Vrinten DH, Gispen WH, Groen GJ, Adan RA, Antagonism of the melanocortin system reduces cold and mechanical allodynia in mononeuropathic rats, J Neurosci., 2000 Nov 1, 20(21):8131-7; Vrinten DH, Adan RA, Groen GJ, Gispen WH, Chronic blockade of melanocortin receptors alleviates allodynia in rats with neuropathic pain, Anesth Analg., 2001 Dec, 93(6):1572-7);

- anxiety or depression as discussed by Chaki et al. (Chaki S, Hirota S, Funakoshi T, Suzuki Y, Suetake S, Okubo T, Ishii T, Nakazato A, Okuyama S, Anxiolytic-like and antidepressant-like activities of MCL0129 (1-[(S)-2-(4-fluorophenyl)-2-(4-isopropylpiperadin-1-yl)ethyl]-4-[4-(2-methoxynaphthalen-1-yl)butyl]piperazine), a novel and potent nonpeptide antagonist of the melanocortin-4 receptor, J Pharmacol Exp Ther., 2003 Feb, 304(2):818-26 ; Chaki S, Okubo T, Melanocortin-4 receptor antagonists for the treatment of depression and anxiety disorders, Curr Top Med Chem. 2007, 7(11):1145-51) ;

- drug addiction as discussed by Alvaro *et al.* and Hsu *et al.* (Alvaro JD, Tatro JB, Duman RS, Melanocortins and opiate addiction, Life Sci., 1997;61(1):1-9 ; Hsu R, Taylor JR, Newton SS, Alvaro JD, Haile C, Han G, Hruby VJ, Nestler EJ, Duman RS, Blockade of melanocortin transmission inhibits cocaine reward, Eur J Neurosci., 2005 Apr, 21(8):2233-42).

The applicants have found that the novel imidazopyridine derivatives of the formula according to the present invention possess a good affinity for the MC4 receptors.
Thus, these results confirm that said compounds can be used for treating pathological states or metabolic diseases chosen among weight disorders (obesity, cachexia, anorexia), mental disorders (anxiety, depression), pain and more particularly neuropathic pain, or sexual activity disorders (erective disorders).

It is declared by the undersigned that all statements made herein of undersigned's own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements made were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under 18 U.S. Code § 1001, and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Dated: This 17 day of July, 2008.

Christophe Thurieau.

Christophe THURIEAU

| Example | Ki (rnMC4) <3.5 µM | Ki (rnMC4) <1 µM | Ki (rnMC4) <250 nM |
|---|---|---|---|
| 2 | | X | |
| 9 | | X | |
| 10 | X | | |
| 24 | X | | |
| 25 | | X | |
| 27 | | X | |
| 32 | X | | |
| 36 | X | | |
| 39 | | X | |
| 43 | | X | |
| 48 | | X | |
| 49 | | X | |
| 50 | | X | |
| 51 | | X | |
| 53 | | X | |
| 55 | | X | |
| 57 | | X | |
| 58 | | X | |
| 61 | X | | |
| 62 | X | | |
| 63 | | X | |
| 64 | X | | |
| 65 | | X | |
| 66 | | X | |
| 67 | | X | |
| 68 | | X | |
| 69 | | X | |
| 70 | | X | |
| 71 | | X | |
| 72 | X | | |
| 73 | | X | |
| 74 | | X | |
| 75 | | X | |
| 76 | | | X |
| 77 | | X | |
| 78 | X | | |
| 79 | | X | |
| 80 | | X | |
| 81 | | X | |
| 82 | X | | |

| Example | Ki (rnMC4) <3.5 µM | Ki (rnMC4) <1 µM | Ki (rnMC4) <250 nM |
|---|---|---|---|
| 83 | X | | |
| 84 | X | | |
| 85 | | X | |
| 86 | | X | |
| 87 | | X | |
| 88 | | X | |
| 89 | | X | |
| 90 | | X | |
| 91 | | | X |
| 92 | | | X |
| 93 | | X | |
| 94 | | X | |
| 95 | | | X |
| 96 | | | X |
| 97 | | X | |
| 98 | | X | |
| 102 | X | | |
| 103 | X | | |
| 104 | X | | |
| 105 | X | | |
| 106 | | X | |
| 107 | | | X |
| 108 | | | X |
| 109 | | X | |
| 110 | | | X |
| 111 | | X | |
| 112 | | | X |
| 113 | | | X |
| 120 | | | X |
| 121 | | | X |
| 122 | | | X |
| 123 | | | X |
| 124 | | | X |
| 125 | | X | |
| 126 | | | X |
| 127 | | X | |
| 128 | | X | |
| 129 | | X | |
| 130 | | X | |
| 131 | | X | |

| Example | Ki (mMC4) <3.5 µM | Ki (mMC4) <1 µM | Ki (mMC4) <250 nM |
|---|---|---|---|
| 132 | | X | |
| 133 | X | | |
| 134 | | X | |
| 135 | | X | |
| 136 | | X | |
| 137 | | X | |
| 138 | | | X |
| 139 | | X | |
| 140 | | X | |
| 141 | | X | |
| 142 | | | X |
| 143 | | X | |
| 144 | | X | |
| 145 | | X | |
| 146 | | | X |
| 147 | | X | |
| 148 | | | X |
| 149 | | X | |
| 150 | | X | |
| 151 | | X | |
| 152 | | X | |
| 153 | | X | |
| 154 | | X | |
| 155 | | X | |
| 156 | X | | |
| 157 | | X | |
| 158 | | X | |
| 159 | | X | |
| 160 | | X | |
| 161 | | X | |
| 162 | | X | |
| 163 | | X | |
| 164 | | X | |
| 165 | | | X |
| 166 | | X | |
| 167 | | | X |
| 168 | | | X |
| 169 | | X | |
| 170 | | | X |
| 171 | | | X |

| Example | Ki (mMC4) <3.5 µM | Ki (mMC4) <1 µM | Ki (mMC4) <250 nM |
|---|---|---|---|
| 172 | | X | |
| 173 | | X | |
| 174 | | | X |
| 175 | | | X |
| 176 | | X | |
| 177 | | X | |
| 178 | | X | |
| 179 | | | X |
| 180 | | | X |
| 181 | X | | |
| 182 | | | X |
| 183 | | | X |
| 184 | | | X |
| 185 | | | X |
| 186 | | | X |
| 187 | | | X |
| 188 | | X | |
| 189 | | | X |
| 190 | | | X |
| 191 | | X | |
| 192 | | X | |
| 193 | | | X |
| 194 | | | X |
| 195 | | X | |
| 196 | | | X |
| 197 | | X | |
| 198 | | X | |
| 199 | | | X |
| 200 | | X | |
| 201 | | X | |
| 202 | | | X |
| 203 | X | | |
| 204 | X | | |
| 205 | X | | |
| 206 | X | | |
| 207 | X | | |
| 208 | X | | |
| 209 | X | | |
| 210 | | X | |
| 211 | | | X |

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